Channel Regulation & Modulation I

527-Pos Board B327
Regulation of Kv1.3 Channels by a Matrix Metalloproteinase
Hai M. Nguyen.
Matrix metalloproteinase 23 (MMP23) contains a functional K^+ channel-blocking toxin domain (TxD) with structural similarity to the sea anemone toxins BgK and ShK (J. Biol. Chem. 2010.285:9124-9136). MMP23 co-localizes with and traps TxD-sensitive Kv1.3 channels intracellularly without affecting TxD-resistant Kv1.2 channels (J. Biol. Chem. 2010.285:9124-9136). Here we use C-terminal deletion analysis to define the segments of MMP23 required for regulation of human Kv1.3. Confocal microscopy showed that eGFP-Kv1.3 co-localized with dsRed-tagged MMP23 and with three deletion constructs of MMP23 (lack the IgCAM domain, IgCAM + TxD, IgCAM + TxD + Catalytic Domain), but not with dsRed (vector control), in COS-7 cells. Patch-clamp experiments revealed suppression of Kv1.3 currents by full-length dsRed-MMP23 and by the deletion construct lacking all three external domains (IgCAM+TxD+Catalytic domain), but not by dsRed. These results indicate that the N-terminal segment of MMP23 (stretching from the N-terminus, through the single transmembrane segment, and ending at the external furin-cleavage site) is sufficient for both current suppression and co-localization with Kv1.3 channels. Western blot analysis demonstrated cleavage of external loops of Kv1.3 by full-length MMP23. Thus, MMP23's N-terminal segment associates with and retains Kv1.3 intracellularly, while the TxD binds to the channel pore and positions the catalytic domain to cleave external loops of Kv1.3.

528-Pos Board B328
Differential Influence of DOPG and DOPA Anionic Phospholipids on Single Channel Conductance of KcsA
Alexander Prokofyev, Vitya Vardanyan, Olaf Pongs.
Binding of anionic phospholipids to non-annular binding site of KcsA has been shown with crystallographic and biochemical methods. To gain insight into the functional importance of these specific protein-lipid interactions we studied the single channel properties of KcsA in planar lipid bilayer by varying the anionic phospholipid content of the membrane. Phospholipids with identical acyl chains and equivalent headgroup charges have been chosen for this purpose. Single channel properties of KcsA were unchanged, when the content of anionic phospholipids was kept below 30% in planar lipid bilayers formed from DOPC neutral phospholipid as remainder component. Upon gradual increase of membrane DOPG content up to 100%, a significant increase in single channel conductance was observed only at positive potentials (from $82{,}2 \pm 1{,}4$ pS to $116{,}3 \pm 0{,}7$ pS at +100mV in symmetrical 150 mM KCl solution). Surprisingly, equimolar increase of DOPA content of the membrane had no influence on single channel conductance of KscA. Differential influence of DOPG and DOPA phospholipids was preserved at varying K+ concentrations of recording solutions ranging from 50 to 250 mM. Using KcsA-Kv1.3 chimera we could show that the stretch of amino acids between E51 and A65 in KcsA was responsible for differential sensitivity of KcsA for DOPA and DOPG. Taken together, our data show that the structure of the phospholipid headgroup is important for phospholipid-specific modulation of single channel conductance in KcsA.

529-Pos Board B329
Dual Regulation of Kv1.2 Activation by PIP2
Aldo A. Rodriguez-Menchaca, **Scott K. Adney**, Diomedes E. Logothetis.
Phosphatidylinositol (4,5)-bisphosphate is now recognized as a ubiquitous regulator of ion channels and transporters. We report here that PIP2 regulates activation of Kv1.2, a Shaker family voltage-gated potassium channel. In inside-out macropatches of Xenopus laevis oocytes, loss of PIP2 induced a leftward shift in the voltage-dependence of activation and a reduction in maximal current, which could be restored by exogenous PIP2 application. These findings were reproduced using the voltage-dependent lipid phosphatase Ci-VSP, which preferentially dephosphorylates PI(4,5)P2 to PI(4)P when activated by depolarization. The dual effect of PIP2 on the voltage-dependence of activation and current level exhibited distinct kinetics. The time course of PIP2 depletion (e.g. during rundown of activity) or recovery (e.g. upon re-phosphorylation of PI(4)P) indicated that the effect on the voltage-dependent shift proceeded with faster kinetics than the current level. Furthermore, high concentrations of diC8-PIP2, a soluble analog of PIP2, partially restored the shift in the voltage dependence of activation caused by PIP2 antibody but failed to do the same for the current level. Taken together, the two effects of PIP2 on the voltage-dependence of activation and the current level of Kv1.2 channels proceeded with distinct kinetics and sensitivity to PIP2, suggesting distinct underlying molecular determinants.

530-Pos Board B330
The Cytoplasmic N-Terminal Domain of DPP6K Disrupts KChiP Modulation of Kv4 Channels
Henry H. Jerng, Paul J. Pfaffinger.
Kv4 channel modulatory subunits, including the cytoplasmic Kv channel interacting proteins (KChIPs) and transmembrane dipeptidyl peptidase-like proteins (DPLPs), have variable N-terminal domains that can dramatically influence Kv4 channel function. These modulatory effects probably underlie the distinctive properties of subthreshold A-type potassium currents (I_{SA}) in different neuron types. In this study we have characterized unique modulatory effects encoded in the N-terminus of DPP6K, a brain DPP6 isoform named for a distinctive N-terminal lysine cluster. In co-expression studies of Kv4.2+KChIP3a+DPP6 ternary channel complexes in *Xenopus* oocytes, DPP6K produces dramatically slower recovery from inactivation (at −100 mV: τ-1 = 84 ms (58%), τ-2 = 324 ms (42%)) when compared to DPP6S (τ = 25 ms)or DPP6a (τ = 10 ms). The slower recovery kinetics produced by DPP6K are accompanied by a hyperpolarizing shift (~ 8 mV) in steady-state inactivation. To explore the functional determinant for these DPP6K effects, we conducted deletion analysis and point mutations on the DPP6K N-terminal domain. The results show that the unique functional effects of DPP6K are not attributable to the distinctive lysine residues but instead depend on hydrophobic residues 12-16. Alanine scanning of residues 12-16 shows that Met-12 and Val-16 are the critical residues for these modulatory effects, with substitution of both residues completely eliminating the effects of DPP6K on inactivation. To examine the mechanism for DPP6K modulation of channel inactivation, we compared the properties of Kv4.2+KChIP3a+DPP6K to Kv4.2/Δ2-40+DPP6K. Surprisingly, Kv4.2/Δ2-40+DPP6K and Kv4.2+KChIP3a+DPP6K channels recover from inactivation similarly and have similar midpoints of steady-state inactivation (~ −70 mV), as if DPP6K blocks the normal modulatory effects of KChIP3a.

531-Pos Board B331
The Two-Pore-Domain Potassium Channels, TASK-1 and TASK-3, regulate Pancreatic Beta-Cell Membrane Potential in Response to pH and Anesthetics
Prasanna Dadi, Louis H. Philipson, **David A. Jacobson**.
Glucose stimulation of the pancreatic β-cell depolarizes the membrane potential to allow activation of voltage dependent calcium channels resulting in action potential (AP) firing, calcium influx, and insulin secretion. Two-pore-domain potassium (K2P) channels regulate the membrane potential from where AP firing occurs in many neurons. However, a role of K2P channels in regulating the pancreatic β-cell membrane potential is unknown and thus we addressed expression and function of the TASK K2P channels in the mouse β-cell. We find that TASK-1 and TASK-3 channels are expressed in mouse β-cells. Furthermore, β-cell TASK-like currents are sensitive to external pH, showing inhibition with acidic pH and stimulation with alkaline pH. Interestingly, glucose regulates β-cell intracellular pH causing acidic conditions in low glucose and alkaline conditions in high glucose, which may serve to regulate β-cell TASK channel activity. Increasing glucose from 2 to 14 mM caused polarization of the β-cell membrane potential whereas reducing glucose from 14 to 2 mM caused membrane depolarization by 6.1 +/- 2.2 mV when K_{ATP} was inhibited with tolbutamide. Similarly extracellular alkalinization (pH 8) resulted in polarization of the β-cell membrane potential by 9.19 +/-2.0 mV, whereas extracellular acidification (pH 6) caused membrane depolarization by 9.84 +/-2.9 mV. Anesthetic compounds that activate (halothane) or inhibit (lidocaine) TASK channels were evaluated for their ability to regulate β-cell membrane potential. Treatment of islets with lidocaine depolarized the β-cell membrane potential by 6.2 +/- 1.5 mV and halothane treatment resulted in β-cell membrane polarization. Both lidocaine and halothane have been shown to cause perturbations in human glucose homeostasis. Thus, this data implicate important roles for TASK channels in regulating the β-cell membrane potential, which may influence anesthetic and pH/glucose induced modulation of insulin secretion.

532-Pos Board B332
Membrane Modulation of KcsA Structural Dynamics and Ion Channel Function
Ryan W. Davis, Steven Branda, Carol L. Kozina, Seema Singh,
Edward G. Moczydlowski, Susan L. Rempe.
The chemical environment presented by the lipid bilayer is hypothesized to have potentially dramatic effects on the structure and function of integral membrane proteins, especially ion channels. Previously, Valiyaveetil, et al. reported that although a variety of phospholipids provide sufficient structural stabilization to refold KcsA monomers into the tetrameric quaternary structure, negatively charged lipids are required to re-establish K-channel